

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C12Q 1/68, G01N 33/569	A1	(11) International Publication Number: WO 93/20231 (43) International Publication Date: 14 October 1993 (14.10.93)
(21) International Application Number: PCT/GB93/00647 (22) International Filing Date: 29 March 1993 (29.03.93) (30) Priority data: 9207069.7 31 March 1992 (31.03.92) GB (71) Applicant (for all designated States except US): THE MINISTER OF AGRICULTURE, FISHERIES AND FOOD IN HER BRITANNIC MAJESTY'S GOVERNMENT OF THE UNITED KINGDOM OF GREAT BRITAIN AND NORTHERN IRELAND [GB/GB]; Whitehall Place, London SW1A 2HH (GB). (72) Inventors; and (75) Inventors/Applicants (for US only) : WOODWARD, Martin, John [GB/GB]; 23 Burnsall Close, Farnborough, Hampshire GU14 8NN (GB). THORNS, Christopher, John [GB/GB]; 11 Lincoln Drive, Pyrford, Woking, Surrey GU22 8RL (GB).	(74) Agent: LOCKWOOD, Peter, Brian; DIPR IPR 1, Room 2002, Empress State Building, Lillie Road, London SW6 1TR (GB). (81) Designated States: AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(54) Title: METHOD OF TESTING FOR SALMONELLA (57) Abstract This invention relates to a method of testing for microorganisms of Salmonella serotypes, particularly <i>Salmonella Typhi</i> (<i>S. Typhi</i>) by detection of nucleic acid sequences related specifically to these serotypes genomic DNA. The invention further provides test kits for performing tests according to this method.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

METHOD OF TESTING FOR SALMONELLA.

The present invention relates to a method of testing for microorganisms of certain serotypes of the genus *Salmonella* by detection of their characteristic polynucleotide sequences. The invention further provides test kits for performing tests according to this method.

Salmonella typhi (*S. typhi*) is an invasive organism causing systemic disease, as opposed to the common salmonella intestinal disease, and is the major cause of enteric fever in humans. Oral ingestion of *S. typhi* organisms results in their invasion of the intestinal epithelium, entry into the circulatory system and colonization of the reticuloendothelial organs. After multiplication at these sites over a week or so they invade the bloodstream and cause septicemia, seeding the intestine through the gallbladder and bile duct, resulting in haemorrhage and perforation of the Peyer's patches in severe cases.

S. typhi organisms are usually recoverable from the blood and urine early in the disease and from the faecal stools between the third and fifth week of illness. It is found that about 2.5% of typhoid patients become chronic gallbladder carriers; those who are elderly or female being more susceptible to this condition. Significantly it is difficult to isolate *S. typhi* from such gallbladder carriers and thus, inter alia, provision of a rapid method for identification of these and patients in the early stages of the disease is desirable.

In the present applicant's copending applications PCT/GB 91/01690 (inventor Thorns) and PCT/GB 91/01691 (inventor Woodward) there are described methods for the detection of certain strains of salmonella by detection of a specific fimbrial antigen which occurs only in *S. enteritidis*, some strains of *S. dublin* and, as so far determined, only one strain each of *S. moscow* and *S. blegdam*; all other strains (several hundred) tested lacked this antigen. Both the latter serotypes which are very closely related to *S. enteritidis*, are extremely rare and have not been seen by the CVL's reference laboratory since the Zoonoses Order (1975) started in the United

Kingdom in 1976. The detection of strains expressing SEFA is therefore an indication of S. enteritidis or S. dublin, and indicates that a strain is not of S. typhi.

The present inventors have now surprisingly found that serotypes of S. typhi also have DNA encoding for this specific fimbrial antigen and thus that methods of copending application PCT/GB 91/01691 can be adapted for the detection of such organisms in a rapid and reliable manner. The antigen encoded is designated hereinafter as SEFA, Salmonella enteritidis Fimbrial Antigen, but the DNA coding for it is common to members of the enteritidis, dublin and typhi serogroups. Characterising data regarding SEFA is provided below, its amino acid sequence being given as SEQ. ID No 2, and the DNA sequence of the double stranded polynucleotide region containing the sequence encoding for it provided as SEQ. ID No 1 and SEQ. ID No 3 in the sequence listing attached hereto. The bases corresponding to the amino acid sequence are bases 73 to 600 of the SEQ ID No 1, corresponding to bases 1788 to 2315 of SEQ ID No 3.

The inventors have further now determined that although the SEFA antigen can be expressed in vitro by S. enteritidis, many S. dublin, and the isolated examples of S. moscow and S. blegdam, expression by S. typhi has so far been impossible to demonstrate, even when incubated in preferred expression supporting media of PCT/GB 91/01690.

Thus the present invention provides a method for testing for the presence of microorganisms of Salmonella serotype S. typhi comprising testing a sample for the presence of a nucleic acid sequence characteristic of genomic DNA from the region encoding for SEFA (SEQ ID No 1 and SEQ. ID No 3) and relating the presence of this to the presence of said serotype. Preferably, the relating of the presence of the characteristic sequence to presence of S. typhi will take into account the nature of the sample, and the likelihood of other SEFA encoding serotypes being present. Provision of further distinguishing steps provides a test for S. enteritidis, S. typhi or S. dublin.

A particular method for determining the presence of polynucleotide sequences characteristic of genomic DNA from the region encoding for SEFA is provided by copending PCT/GB 91/01691 and comprises use of polynucleotide hybridization probes targeted to identify such sequences using techniques which are well known in the art. Examples of such probes are also exemplified below.

A further such method for determining the presence of such characteristic polynucleotide sequences comprises subjecting a sample of analyte to conditions under which polynucleotide sequences are replicated by use of specific sequence amplification reaction, for example the ligase or polymerase chain reactions (LCR and PCR), as provided by copending PCT/GB 91/01691. Such PCR amplification is well known in the art and suitable primers for use in PCR targeted at the sequences characteristic of SEFA are exemplified herein below.

The production of product in the presence of specific primers can be taken as a positive indication of the presence of the target sequence but it is possible to cross check this result by determination of further specific hybridization using probe sequences, such technique being well known in the art, using suitably different sequences selected from those exemplified for primer purposes. Methods for carrying out the PCR are conventional and illustrated by those described in EP-A-0201184 and EP-A-0200362 (both Cetus Corp).

Specific conditions for carrying out PCR on cells or DNA/RNA as provided in aqueous analyte samples are exemplified in EP 0409159 (Shimadzu) and EP 0438115 (Perkin Elmer), but variations of these will be known to a person skilled in the art of PCR based detection methods. The aqueous samples to which the present probes will be applied will typically be bodily fluids or food, faecal or tissue samples (eg. homogenates) but may potentially be any material from which cells or polynucleic acids may be derived.

As will be clear to those skilled in the art, it will not be necessary to employ probes and/or primers which are targeted at the whole of

this coding sequence in order to ascertain its presence. As is conventional in the art, probe and primer sequences can be targeted at any characteristic sequence, preferably being of 12 or more bases long, more preferably 16 or more bases long, in so far as these are statistically unlikely to be found in interfering, non-targeted sequences. Thus preferred probes and/or primers consist of at least any contiguous 12, preferably any contiguous 16 bases of either of the respective strands of SEQ ID No 1 or SEQ ID No 3, preferably 73-600 of SEQ ID No 1 or its complement; primers obviously running 5'-3'.

It will be realised that an analyte determined to contain the SEFA characteristic sequences referred to above might equally comprise DNA/RNA or microorganisms of the other serotypes, most likely S. enteritidis or S. dublin. However, in practice, when analytes are provided they will be derived from or related to subjects showing symptoms which will be consistent with either S. typhi or S. enteritidis/S. dublin, but not both and thus a test may be carried out with a degree of certainty that a positive result will be indicative of the salmonella consistent with the particular symptoms. However, in the event that there are no symptoms to assist analysis (eg. where no live subject is available or where the samples are not directly derived from a human or animal body) or where it is desired to be more unequivocal in determination of the organisms present, the present invention further provides a method for determining the presence of an organism having DNA or RNA characteristic of that region encoding for SEFA and also determining its identity as being S. typhi, S. enteritidis or S. dublin comprising

- (a) testing a sample of material under investigation for the presence of a nucleic acid sequence characteristic of the genomic DNA from the region encoding for SEFA (as SEQ. ID No 1 or SEQ ID No 3);
- (b) testing that or a further sample of the material for the presence of an antigenic amino acid sequence or antibody thereto or a genomic nucleic acid sequence; said amino acid sequence or nucleic

acid sequence being associated with one or more of the S. enteritidis, S. dublin or S. typhi serotypes, but not found in all three;

and relating the results of tests (a) and (b) to the presence or absence of each of the three serotypes.

Thus step (a) may be carried out using any sequence determined to be characteristic of the genomic DNA of the region encoding for SEFA (as provided in SEQ ID 1 or SEQ ID No 3 below); these preferably being a sequence of sufficient length to provide statistical probability of correct identification, ie. conventionally any sequence of 12 or more contiguous bases, more preferably of 16 or more bases, selected from this sequence.

Preferably the step (a) is carried out by use of specific sequence amplification, preferably PCR, and most preferably by carried out by use of PCR primers described below (SEQ ID 4, 5, 6, 7, 8, 9 and 10). Similarly these primers may be effectively employed as hybridization probe sequences when used in labelled form. Step (b) conveniently may be carried out using any distinguishing test utilising the differences in amino acid sequences characteristic of the particular serotypes.

As disclosed in PCT/GB 91/01690, S. enteritidis and S. dublin may be conveniently distinguished antigenically using a polyclonal antibody raised to S. dublin which is then absorbed with S. enteritidis in order to remove any antibody which crossreacts with the two. In this way a polyclonal antibody reactive with S. dublin is left that is not reactive with S. enteritidis. Similarly such polyclonal antibodies which are specific, as between the three 'common' SEFA encoding serotypes enteritidis, dublin and typhi, may be prepared by cross absorbing non-specific antibodies onto the two serotypes to which the antibodies were not raised in order to leave the desired characterising antibodies. Cross-absorption with the other two isolated strains of S. moscow and/or S. blegdam will also be advantageous, as will provision of specific antibodies for these.

More preferably monoclonal antibodies will be prepared using standard techniques to provide producing hybridoma cell lines such that antibodies all with the same target sequence and affinity may be used for the determinative tests. Of particular use are antibodies raised to the SEFA antigen or an epitope of that, in so far as these will bind to SEFA as expressed by all the strains of interest, except S. typhi, thus providing a ready check as to identity of the organism. These antibodies are subject of PCT/GB 91/01960 as described previously and hybridoma cells expressing one of these has been deposited in accordance with the Budapest Treaty at the European Collection of Animal Cell Cultures, PHLS Centre for Applied Microbiology & Research, Porton Down, Salisbury, WILTS SP4 OJQ, under accession number 90101101 on 11th October 1990.

In all cases of use of antibodies as reagents in these tests it will be possible to enhance visualisation of the antibody-antigen binding phenomena by labelling them with coloured latex particles as is known in the art (Hechemy K E and Michaelson (1984) Lab Management 22 27-40). Alternativley use may be made of secondary antibodies as is also known in the art whereby the secondary antibody is targeted at the antibody reagent and is labelled, eg. with gold, such that when excess unbound antibody is washed away, eg. so as to be removed from cells or fimbria in the sample, it is possible to observe the gold or other label clustered around the cells or fimbria thus indicating antibody binding on these.

As further explained in PCT/GB 91/01690, it is found that the content of the culture medium is a crucial factor in the in vitro production of SEFA epitopic sites on the Salmónella fimbria of the SEFA expressing strains. Peptone water and Enriched E broth (see Francis et al (1982) J. Clinical. Microbiol.. 15: 181-183) are examples of liquid media which will support relatively weak expression in reliable manner. Solid media examples include desoxycholate citrate agar, McConkey agar, Nutrient agar, Salmonella Shigella agar, Sheep blood agar, Xylose Lysine descholate. For more reliable and/or sensitive testing it may be necessary to use a medium that is more potent in supporting the expression; examples of such media being Oxoid

Isosensitest and Sensitest agars. Suitable media may be selected for the ability to support SEFA expression by S. enteritidis as determined by antigen-antibody binding assay using one of the monoclonal antibodies, eg. that deposited as referred to above or other SEFA derived specific antibodies.

The present invention further provides kits for performing the methods of this invention; kits for the determination of the presence of SEFA expressing organisms or their SEFA associated components (ie. SEFA or an epitopic part thereof, antibodies to SEFA or epitopic parts thereof, polynucleotides encoding for SEFA or epitopic parts thereof) being already provided by the aforesaid copending patent applications.

Thus kits of the present invention are characterised in that in addition to reagents for the determination of the presence of these SEFA encoding region characteristic sequences they, further comprise reagents for the determination of the presence of components distinctive of one or two of the three SEFA serotypes S. enteritidis, S. dublin and S. typhi such that the presence of each of these can be ascertained. Kits further containing recognition reagents for the S. moscow and/or S. blegdam are also provided for use on samples where the presence of these is a possibility.

The reagents for the determination of the presence of the SEFA encoding region characteristic polynucleotide sequences will comprise at least one or both of the following:

(i) specific sequence amplification reaction primers targeted at a nucleic acid sequence characteristic of the genomic DNA region encoding for SEFA (ie. characteristic of SEQ ID No 1 or SEQ ID No 3), and capable of initiating specific sequence amplification reaction production of these sequences in the presence of enzymes, such as polymerases or ligases;

(ii) hybridization probes targeted at characteristic polynucleotide sequences of the region encoding for SEFA (ie. of SEQ ID No 1 or SEQ

ID No 3) and capable of selectively hybridizing with these under test conditions.

Preferred kits comprising reagents for distinguishing a strain as being S. typhi may comprise antibodies capable of identifying one or more, but not all, of the SEFA encoding strains, or hybridoma cells capable of producing these. For example, the deposited cells referred to above or their antibodies which are optionally in labelled form (as is understood in the art), more preferably immobilised on solid carriers. Alternatively the kits contain labelling agents such as latex particles which may be coloured, for conveniently use.

The SEFA expression supporting media of PCT/GB 91/01960 may also be included for identifying the expressing strains, and thus providing evidence that S. typhi is not the strain present. Examples of preferred media capable of supporting SEFA expression are sold by Oxoid under the trade name 'Oxoid Sensitest Agar' and 'Oxoid Iso-Sensitest Agar'. The use of these media is described in PCT/GB 91/01960.

Culture of the *Salmonella* micro-organisms on the medium is by entirely standard conditions, eg by incubation at about 37°C until a sufficient number of the micro-organisms having epitopic sites on their fimbriae have grown, for example typically by overnight incubation. An incubation temperature of above 22°C is preferred for the effective production of the SEFA bearing fimbriae. In applying the test in practice, a sample of the analyte eg. a suspected bodily material would be taken, containing a cross-section of all the micro-organisms present in the material, and these would then be cultured on the medium so that the salmonella, if present, grows among any other micro-organisms that might be present. The presence of other micro-organisms does not seem to adversely affect the test.

Procedures for raising both polyclonal and monoclonal antibodies to salmonella surface antigens are well known. Thus, for example, S. enteritidis may be grown on a medium as described above so that

antigenic fimbriae are produced, these then may be used to immunise mice from which spleen cells are subsequently isolated and fused with a myeloma cell line to form hybridomas. These hybridomas may then be seeded into microwells and monitored for antibody production, eg by ELISA or a similar technique. Antibody-producing hybridomas may then be cloned to produce a mouse monoclonal antibody to the Salmonella fimbrial antigen. MABs may be produced by the known method of intraperitoneally injecting hybridoma 10 cells (eg; 10^6) into mice and withdrawing ascites after 20 days; this can be used in crude form if necessary.

The exemplified monoclonal antibodies use further extends to (i) the determination of media suitable for growing salmonella possessing the required antigenic fimbriae and (ii) for identification of said antigenic fimbriae and antigens comprising the SEFA epitope itself. Thus further specific media suitable for the performance of the method of the invention may be easily identified by screening salmonella grown in them for the ability to produce immunoagglutination with said MABs; a positive result indicating a suitable medium. Either the whole Salmonella micro-organisms (live or dead) or a part thereof which includes the fimbrial antigen with the SEFA epitopic site may be detected by the antibody. In the latter case methods are well known, eg. mild heat shock treatment at 60°C for 30 minutes, for detaching fimbriae from salmonella micro-organisms, and isolation of the fimbrial antigen in this way should lead to a more specific test result.

The epitopic sites employed in this method are present on a fimbrial structure produced on the surface of S. enteritidis grown on the media described above and in vivo, which is less than 6 nm in diameter and consists of identical repeating subunits each of molecular weight between 14,000 and 15,000. These fimbriae have a 'kinked' conformation such that they entangle and extend in a matted form to approximately 200nm from the cell surface. By applying size exclusion HPLC and SDS-PAGE to the fimbrial antigen isolated in such a way it has been determined that the SEFA antigenic protein employed

appears to have a molecular weight of approximately 14,300. Isolated SEFA as described here has a major antigenic activity and its amino acid sequence is given below.

In use of SEFA or an epitopic part thereof to determine the presence of SEFA antibodies in an analyte sample, the antigen in the form of whole micro-organisms, the isolated fimbriae or isolated SEFA or epitopic part thereof may be immobilised on a substrate such as a microtitre plate well, using known methods. This immobilised antigen may be exposed to a solution suspected of containing the SEFA antibody, then after washing a second labelled antibody capable of binding to the unlabelled SEFA antibody may be applied (eg: a labelled anti-human Ig G) to the wells. After further washing detection of binding between this second antibody and the SEFA antibody itself bound to the immobilised antigen may then be observed by the presence of the bound label on the well. Other antibody/second antibody combinations will occur to the man skilled in the art (eg murine bovine or chicken antibodies/anti-murine anti-bovine or anti-chicken second antibodies).

In a yet further way the antibody may be immobilised on a substrate and the immobilised antibody may then be exposed to a solution containing the antigen in the form of for example whole micro-organisms, the isolated fimbriae or the antigenic protein (SEFA), together with an agent capable of competing with the antigen for binding sites on the antibody. The quantity of the agent binding to the immobilised antibody may then be determined, eg: by use of known, labelling techniques. For example the competing agent may be a labelled anti-mouse IgG if the antibody is a mouse monoclonal, or may be labelled fimbrial antigen. The labels used in the above methods may be entirely conventional, and ways of labelling antibodies are well known.

The test kits may contain further reagents and other items for performance of the two or more determinations necessary (ie. the SEFA determination and the serotype enteritidis /dublin/typhi

determination). For example as well the antibodies and the SEFA expression medium, visualising agents and standard result cards may be included. Depending upon the way in which the test is to be applied the antibody may be provided in the form of a solution, eg, for immunoagglutination or if the antigen is to be immobilised, or the antibody may be provided in the aforementioned immobilised form. The test kit may optionally also contain a further antibodies for further cross-checking salmonella serotype, instructions and appropriate vessels for carrying out the test.

In the method of the present invention the presence of nucleic acid sequences characteristic of the genomic DNA from the region encoding SEFA, as defined by SEQ ID No 2 in the listing below, and preferably of the 527 basepair DNA sequence actually encoding for SEFA, as illustrated in SEQ ID No 1, is used to determine the presence of one of the aforesaid salmonella serotypes, and the serotype is further differentiated to determine the presence of S. typhi or one of the expressing strains.

The presence of one of the SEFA encoding region serotypes can be ascertained by use oligonucleotide probes and primers for the purpose of detecting SEFA encoding serotype genomic DNA. As well as the key characteristic sequences so defined sequences encoding for allelic variants of SEFA will also be characteristic of such serotypes.

The polynucleotide sequence directly corresponding to SEFA is on the upper strand as shown above beginning ATGCTAATAG and ending GTATCAAAC in SEQ ID No 1. PCT/GB 91/01691 provides recombinant DNA, plasmids and methods using them for genetically engineering organisms capable of expressing SEFA.

That patent further provides methods for determining the presence of microorganisms having DNA or RNA polynucleotide sequence encoding for SEFA or an epitopic part thereof, allelic variants of these, or such DNA or RNA itself, comprising: (a) providing a sample suspected of containing said encoding polynucleotide sequence; (b) determining the

presence of said sequence by monitoring hybridization of SEFA targeted polynucleotide probes to it.

Such hybridization technique is carried out by methods that are now conventional in the art, using probes which are comprised of sequences complementary to a significant part of the target sequence and using temperature conditions suitable to achieved a desired stringency dependent on the degree of match of the probe to the target. Probes complementary to the length up to the entire target sequence may be used.

Conveniently the characteristic sequence is detected, in both amplified and unamplified tests, by use of a hybridization probe suitably specific thereto which comprises any of the aforementioned sequences, more specifically being one of the sequences in a suitably labelled form eg. being labelled in some way as will be known to a man skilled in the art. Most conveniently the label will incorporate radioactive phosphorous (^{32}P). A preferred such method comprises a PCR step (b) which employs primer pairs targeted to amplify characteristic sequences, a particularly preferred method comprises use of one primer selected from groups (A) and the other from group(B):

Group A(SEQ ID No):

A4: 5' -GTGCGAATGCTAATAGTTGA- 3'
A5: 5' -TGCGTAAATCAGCATCTGCA- 3'
A6: 5' -TCTGCAGTAGCAGTTCTTGC- 3'
A7: 5' -GCTCAGAATACAACATCAGCCAA- 3'

Group B(SEQ ID No):

B8: 5' -AAAACAGGCTGTCTTGTCCA- 3'
B9: 5' -TTAGCGTTTCTTGAGAGCTGG- 3'
B10: 5' -TTTTGATACTGCTGAACGTAG- 3'

These are designated SEQ ID No 4 to 7 (A4-A7) and SEQ ID No 8 to 10 (B8-B10) respectively in the listing below.

Any of the possible pairs selected in this way will bind with the characteristic sequences sufficiently specifically enough for serotype determination purposes using PCR under standard conditions, ie: for determination of a salmonella as being a SEFA encoding serotype and thus of one of the serotypes listed above.

As will be understood by a man skilled in the art, sequences which will specifically hybridize with the characteristic sequences will include those sequences themselves, those having high eg.75% or more, preferably 90% or more conformity to such sequence, and sequences comprising either strand of the two complementary sequences of any of these. Thus the step (c) of the method of this aspect of the invention may be carried out using a variety of hybridization probes that combine sufficiently specifically with the characteristic 'target' sequence. For most purposes the primer sequences selected from those of groups (A) and (B) will be sufficiently specific to give reliable determination of the characteristic sequence, especially if a different 'primer' sequence is used for the probe of step (c) than those used for step (b).

In this way substantially larger quantities of the DNA sequence may be made from the small quantities which may be available by isolation from the S. enteritidis, S. dublin or S. typhi thus increasing the amount of sequence available to be detected. The mere presence of increased amount of DNA may be used in this case to signify presence of target sequence.

Comparison of S. typhi and S. enteritidis DNA. In order to demonstrate the similarity between the SEFA encoding regions of genomic DNA from S. enteritidis and S. typhi, the following experimental was carried out.

1. Genomic DNA was extracted from S. typhi Ty21a using a gentle lysis protocol described by Goldberg and Ohman (1984 J. Bact 158 1115-1121) and resuspended in TE (10mM Tris pH 8, mM EDTA) to a final concentration of 2µg µl⁻¹.
2. Samples of S. typhi DNA (5µg) were denatured by adjusting to pH 2.5 by addition of 1M NaOH to give a final concentration of 0.3M. the DNA sample was spotted onto nylon filters, allowed to air dry and the DNA fixed by UV irradiation.

3. samples of positive control DNA from S. enteritidis and negative control DNA from E. coli were treated as in 2. above and spotted onto the same filter in distinct locations. 4. A 583 basepair DNA fragment encoding the entire SEFA fimbrial antigen removed as a DraI excision product was radiolabelled using ^{32}P by the random hexanucleotide labelling system (Amersham) as described by Feinberg and Vogelstein (1983, Analytical Biochem. 132, 6-13). The labelled DNA was used in DNA:DNA hybridization experiments using the filter prepared in 2. and 3. above following standard methods Woodward and Sullivan, 1991 J. Gen. Micro. 137, 1101-1109). Post hybridization washes were at 65°C with 0.1 x 3SO/0.1% SDS as the final wash solution. 5. Washed filters were air dried and then exposed to X-ray film (Fuji-RX. RTM). Exposed film was developed using standard photographic procedures. DNA from S. enteritidis and S. typhi bound the probe with equal activity while the negative control DNA did not bind it at all.

6. Total genomic DNA (5µg) extracted from S. typhi Ty21a was digested to completion with various restriction endonucleases in separate experiments. Digested DNA was fractionated by standard gel electrophoresis, Southern blotted and hybridized as above (4. and 5.) Unique DNA bands bound the probe.

7. S. typhi genomic DNA (100ng) was used in PCR experiments with the oligonucleotide primers of groups A and B as herein described. In each experiment, using standard conditions (Taiki et al. 1985, Science 230: 1350-1354) amplified products of the desired size were obtained and each product was shown by Southern hybridization to be homologous with the 583 base pair probe.

Thus experiments 1. to 7. demonstrate the common SEFA encoding sequence as being present in S. typhi as well as the previously determined presence in S. enteritidis and S. dublin.

Thus when seeking to differentiate the three SEFA serotypes by use of a polynucleotide sequence encoding for a specific antigenic amino-acid

sequence associated with one or two of them, but not all three it is clear that SEFA is likely to be of no utility. Conveniently sequences will thus be those for the p and G antigens referred to above in the immunological tests.

The various aspects of the invention will now be described by way of the following non-limiting protocol examples.

EXAMPLE 1: Test kit reagents and protocol for use:

A kit for determination of presence of S. typhi is provided as follows. Probes/primers are selected from groups (A) and (B) above for use with standard laboratory reagents for hybridization probing and/or PCR reaction. Such probes may be purchased to order from companies such as Pharmacia UK, or synthesized according to standard techniques (see Gait M J (Edit.) 'Oligonucleotide synthesis- a practical approach', IRL Press, Oxford (1984) and Beaucage & Carruthers, Tetrahedron Letters 22 p1859-1862). Radiolabelling and Southern blotting are carried out by conventional methods.

Kit reagents provided for the purpose of identifying the SEFA encoding strain as of serotype S. typhi, or as one of the SEFA expressing strains, comprise monoclonal antibody directed at SEFA, a polyclonal antibody directed to S. dublin flagella p antigen but not immunoreactive with enteritidis or typhi, a polyclonal antibody directed at G component of S. dublin and S. enteritidis flagella but not immunoreactive with S. typhi, reader cards and preferred growth medium optionally with any of the reagents (eg latex particles) below used in the test.

Coating of latex: To prepare a batch of latex coated with any of the antibodies. Materials: Glycine buffered saline (GBS as above), Bovine serum albumen (fatty acids free) (Code A-6003, Sigma Chemicals), coloured latex (colour chosen to identify a particular antibody on its surface), 0.8microns, 10% suspension (Code K080, Estapor, Rhone-Poulenc), antibody containing fluid, Glass container of

the suitable size - Pressmatic dispenser (Bibby) - Dropper bottles - Labels - Rocking device

Method: volumes of latex, antibody and GBS appropriate for that batch size are mixed in a glass container and incubated for 2 hours at 37°C with constant gentle rocking, centrifuged for 20 minutes at 3500 rpm. The supernatant is discarded and the latex resuspended in appropriate volume of GBS containing 0.1% BSA ready for use. Control latex may be prepared by coating with normal mouse serum collected from 8-12 week old Balb/c mice.

Positive control SEFA, p protein (re dublin) or G component (re enteritidis and dublin) is/are preferably included in the kit or a sample of a salmonella capable of producing them may be provided with a suitable media for enabling SEFA expression for control test purposes.

EXAMPLE 2: Use of test kit of Example 1.

Samples testing positive, as including SEFA coding region characteristic sequences in tests using probes or PCR amplification, were designated as containing organisms of the S. enteritidis/S. dublin expressing type or of S. typhi, and thus put forward for differentiation by the following.

Samples were exposed to test latexes as prepared above are compared with controls using reader cards. One test latex is used to identify presence of SEFA bearing materials (eg; whole organisms). The further test latexes are used to differentiate S. enteritidis, S. dublin and S. typhi. In this protocol S. typhi does not react with any of the antibodies, although other protocols using positive identification will occur to those skilled in the art. The control latex aids determination of false positives caused by, inter alia, autoagglutination. The positive control and reader cards are used to determine degree of response.

Note; other commercially available antisera are available which are capable of differential binding with these three significant SEFA expressing serotypes; for example: Northumbria Biologicals Ltd UK -Pasteur Products- supply:

Product number	Antisera	Distinguishes
2061121	Monovalent H antisera g.m	enter' from dublin
2061117	Monovalent H antisera m	enter' from dublin+typhi
2061118	Monovalent H antisera p	dublin from enter'+typhi

Further such antisera are available from Wellcome Reagents Ltd and are coded in their AL/ZD codes: AL47,48,49/ZD13,14,15.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: THE MINISTER OF AGRICULTURE FISHERIES & FOOD
IN HER BRITANN
(B) STREET: WHITEHALL PLACE
(C) CITY: LONDON
(E) COUNTRY: GB
(F) POSTAL CODE (ZIP): SW1A 2HH

(A) NAME: MARTIN JOHN WOODWARD
(B) STREET: 23 BURNSALL CLOSE
(C) CITY: FARNBOROUGH
(D) STATE: HAMPSHIRE
(E) COUNTRY: GB
(F) POSTAL CODE (ZIP): GU14 8NN

(A) NAME: CHRISTOPHER JOHN THORNS
(B) STREET: 11 LINCOLN DRIVE
(C) CITY: PYRFORD, WOKING
(D) STATE: SURREY
(F) POSTAL CODE (ZIP): GU22 8RL

(ii) TITLE OF INVENTION: METHOD OF TESTING FOR SALMONELLA (iii)

NUMBER OF SEQUENCES: 10

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS (D) SOFTWARE:
PatentIn Release £1.0, Version £1.25 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: GB 9207069.7 (B) FILING DATE:
31-MAR-1992

(2) INFORMATION FOR SEQ ID NO: 1:

(1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 2387 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Salmonella enteritidis/Salmonella typhi

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 73..600

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206197 A1

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 1: FROM 1 TO 2387

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206198 A

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 1: FROM 1 TO 2387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GATCCTTGTT TTTTTCCTTA AATTTTAAAT ATGGCGTGAG TATATTAGCA TCCGCACAGA	60
TAAATTGTGC GA ATG CTA ATA GTT GAT TTT TGG AGA TTT TGT AAT ATG	108
Met Leu Ile Val Asp Phe Trp Arg Phe Cys Asn Met	
1 5 10	
CGT AAA TCA GCA TCT GCA GTA GCA GTT CTT GCT TTA ATT GCA TGT GGC	156
Arg Lys Ser Ala Ser Ala Val Ala Val Leu Ala Leu Ile Ala Cys Gly	
15 20 25	
AGT GCC CAC GCA GCT GGC TTT GTT GGT AAC AAA GCA GAG GTT CAG GCA	204
Ser Ala His Ala Ala Gly Phe Val Gly Asn Lys Ala Glu Val Gln Ala	
30 35 40	
GCG GTT ACT ATT GCA GCT CAG AAT ACA ACA TCA GCC AAC TGG AGT CAG	252
Ala Val Thr Ile Ala Ala Gln Asn Thr Thr Ser Ala Asn Trp Ser Gln	
45 50 55 60	
GAT CCT GGC TTT ACA GGG CCT GCT GTT GCT GCT GGT CAG AAA GTT GGT	300
Asp Pro Gly Phe Thr Gly Pro Ala Val Ala Ala Gly Gln Lys Val Gly	
65 70 75	
ACT CTC AGC ATT ACT GCT ACT GGT CCA CAT AAC TCA GTA TCT ATT GCA	348
Thr Leu Ser Ile Thr Ala Thr Gly Pro His Asn Ser Val Ser Ile Ala	
80 85 90	
GGT AAA GGG GCT TCG GTA TCT GGT GGT GTA GCC ACT GTC CCG TTC GTT	396
Gly Lys Gly Ala Ser Val Ser Gly Gly Val Ala Thr Val Pro Phe Val	
95 100 105	
GAT GGA CAA GGA CAG CCT GTT TTC CGT GGG CGT ATT CAG GGA GCC AAT	444
Asp Gly Gln Gly Gln Pro Val Phe Arg Gly Arg Ile Gln Gly Ala Asn	
110 115 120	
ATT AAT GAC CAA GCA AAT ACT GGA ATT GAC GGG CTT GCA GGT TGG CGA	492
Ile Asn Asp Gln Ala Asn Thr Gly Ile Asp Gly Leu Ala Gly Trp Arg	
125 130 135 140	
GTT GCC AGC TCT CAA GAA ACG CTA AAT GTC CCT GTC ACA ACC TTT GGT	540
Val Ala Ser Ser Gln Glu Thr Leu Asn Val Pro Val Thr Thr Phe Gly	
145 150 155	
AAA TCG ACC CTG CCA GCA GGT ACT TTC ACT GCG ACC TTC TAC GTT CAG	588

TATCCAACAA TACGTGGTAT TGCGAAAACC AATGCGACTG TAGAAGTAAG AAAAAATGGA 2320
 TACTTGATAT ATTCTACTTC AGTCCCCCCC GGGCAATTCT AGATAGGTAG AGAACAAATT 2380
 CTGATC 2387

2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 176 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Met Leu Ile Val Asp Phe Trp Arg Phe Cys Asn Met Arg Lys Ser Ala
 1 5 10 15
 Ser Ala Val Ala Val Leu Ala Leu Ile Ala Cys Gly Ser Ala His Ala
 20 25 30
 Ala Gly Phe Val Gly Asn Lys Ala Glu Val Gln Ala Ala Val Thr Ile
 35 40 45
 Ala Ala Gln Asn Thr Thr Ser Ala Asn Trp Ser Gln Asp Pro Gly Phe
 50 55 60
 Thr Gly Pro Ala Val Ala Ala Gly Gln Lys Val Gly Thr Leu Ser Ile
 65 70 75 80
 Thr Ala Thr Gly Pro His Asn Ser Val Ser Ile Ala Gly Lys Gly Ala
 85 90 95
 Ser Val Ser Gly Gly Val Ala Thr Val Pro Phe Val Asp Gly Gln Gly
 100 105 110
 Gln Pro Val Phe Arg Gly Arg Ile Gln Gly Ala Asn Ile Asn Asp Gln
 115 120 125
 Ala Asn Thr Gly Ile Asp Gly Leu Ala Gly Trp Arg Val Ala Ser Ser
 130 135 140
 Gln Glu Thr Leu Asn Val Pro Val Thr Thr Phe Gly Lys Ser Thr Leu
 145 150 155 160
 Pro Ala Gly Thr Phe Thr Ala Thr Phe Tyr Val Gln Gln Tyr Gln Asn
 165 170 175

2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2387 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

i) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Salmonella enteritidis*/*Salmonella typhi*

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206197 A1

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 3: FROM 1 TO 2387

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206198 A

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 3: FROM 1 TO 2387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GATCAGCAAT TTGTTCTCTA CCTATCTCGA ATTGCCCGGG GGGGACTGAA GTAGAATATA	60
TCAAGTATCC ATTTTGTCTT ACTTCTACAG TCGCATTGGT TTTCGCAATA CCACGTATTG	120
TTGGATAATA TGTCTCTGT GAGAAAGGTA TCATACTTTC ATCTTTATTT AAAGAAAAGC	180
CTCTAAATGG TACGCTATCG AATAAATCTG CACTGGTATA TTTGTCCCA ACTGTTAGTT	240
TGCTCTTTAT TTTTTTAAA CCTCGCTCAG CATAAATATA TGCTGATTCA AATTTTTTTT	300
CGCTTGACAA GTTTGTGACAA GATGATAGAT TCCTTAGCCG CCAGGGACCA AAATTAAAC	360
CAAGGTGAA TTTGACCAA GTATGAATCT CTCTCCTCCA ACCTTAGAAT GCAAATAATT	420
AAGCTCTGTA ATTTAAAAGA AAAGCGTTAA TGCCATCATC CCAGATATTT TCATCAGCAA	480
TTTCACTGTC TATTGGAGAT AGAATTTTAG ATGGTGCAIT TAAAAGCAAA CTTTGGTTAG	540
CCGCGTTATA AGTATATTTA AAGAGGAGAA TGCTCTAAGA GAACGCATTG TTCATTATCA	600
AACTGCAACC CATGAAGCTC CTGGATGGCG ATCCCATACT TTATAAGTTT TTCTTTTGTT	660
AAGCATGGTT CTAGAGTCTG AGTCATTGAT TTGAAATACA ACTCAGTAGT TTCTTTTAAC	720
TGATTATTCA AATATACATC AACACATAA TTACCAGGGA AGCGTTTCAA CACTTAGAAA	780
AGATGCATTG TCACCGGAG AAGACTCAAA CTTCATAGT CGAAATTATA TTGTCTGGAG	840
AAAACATTTT CAGAGTGAAA TACACTGGTT AAAACAAATA GAGTAATTGT GGTTTTCTTC	900
ATTTGTTTTA CACCTTAAAC TAATAATTCT CTTATAATTT CAGCGCCGTT ATCATCCAAA	960
ATAGTCAAAG TTATTTTACT ATGTACTCCA CCAGGTAATT GAGCATATCC AAATGGCTCA	1020

ATATATCCAT TTGGAATATT AAAACTTAAA TTACCAGATT TAATATTTGC AATATTCACA	1080
TATGATGATG TTGGATTCTT TATAACTATA CTATTTCCCTT TTCTCTCTAA CTTTAATTTA	1140
TCTGCAATCT CCATTGTCGT TAAGTCTATA GTTTTAGGCC TATAAATTAA TTTAATACAA	1200
CTATTCGTAA CCACATTCAC ATTAAGATTC GTAGTTATGT TGTTTTTATT ATTGCTTTCA	1260
TTATCATTTA GTGGTGGGAC TCCTTTTACA CACAACCAAT ACAAAGACTC CTCATTTTTA	1320
TTGAATAGAT TACTTGTTGG TATTACCTTC AATCTTGTTT CCGCATTACT TTCAACTTTC	1380
AAAATAGGTG GTGTTACAAT AAATGGAGCT TTTGATGATT TATCATCATT ATATACTTGA	1440
GTTTGAACAA GGATTGGATA TTCTTTTTCA TTCATAATCC AAAAATTGT TGATGGAGCA	1500
TCTTCTTTAT AAATAACACG TGTAGTGCCC AATCTTAGGC CATAATATTT TTGTGTTAAT	1560
AATGGTTGCT CAATTTTTTT ACTTTCCGAA GAAGCAATTG GAAGGTAGCA AAAAAAGGA	1620
AAAAGATAAC AGTTCTACGT ATAAATTTAT TCAAAATATA CATAATCCCT CTTTAAGTTT	1680
TTGCATGGAA GCATAAAACC TATCTATTCA AAATAGATAT TTTTAAATA ATAAATTAT	1740
CCAACTCGC TCATATTGAG GGCATTTATA AAGTTTAAAT TAAATTAGTT TTGATACTGC	1800
TGAACGTAGA AGGTCGCAGT GAAAGTACCT GCTGGCAGGG TCGATTTACC AAAGGTTGTG	1860
ACAGGGACAT TTAGCGTTTC TTGAGAGCTG GCAACTCGCC AACCTGCAAG CCCGTCAATT	1920
CCAGTATTTG CTTGGTCATT AATATTGGCT CCCTGAATAC GCCCAGGAA AACAGGCTGT	1980
CCTTGTCAT CAACGAACGG GACAGTGGCT ACACCACCAG ATACCGAAGC CCCTTTACCT	2040
GCAATAGATA CTGAGTTATG TGGACCAGTA GCAGTAATGC TGAGAGTACC AACTTTCTGA	2100
CCAGCAGCAA CAGCAGGCCC TGTAAGCCA GGATCCTGAC TCCAGTTGGC TGATGTTGTA	2160
TTCTGAGCTG CAATAGTAAC CGCTGCCTGA ACCTCTGCTT TGTTACCAAC AAAGCCAGCT	2220
GCGTGGGCAC TGCCACATGC AATTAAAGCA AGAACTGCTA CTGCAGATGC TGATTTACGC	2280
ATATTACAAA ATCTCCAAA ATCAACTATT AGCATTCGCA CAATTTATCT GTGCGGATGC	2340
TAATATACTC ACGCCATTTT AAAAATTTAA GAAAAAAAC AAGGATC	2387

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Salmonella enteritidis/Salmonella typhi

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206197 A1

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 4: FROM 1 TO 20

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206198 A

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 4: FROM 1 TO 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

GTGCGAATGC TAATAGTTGA

20

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Salmonella enteritidis/Salmonella typhi

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206197 A1

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 5: FROM 1 TO 20

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206198 A

(I) FILING DATE: 01-OCT-1991

(J) PUBLICATION DATE: 16-APR-1992

(K) RELEVANT RESIDUES IN SEQ ID NO: 5: FROM 1 TO 20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGGCGTAAATC AGCATCTGCA

20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Salmonella enteritidis/Salmonella typhi
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 9206197 A1
 - (I) FILING DATE: 01-OCT-1991
 - (J) PUBLICATION DATE: 16-APR-1992
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 6: FROM 1 TO 20
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 9206198 A
 - (I) FILING DATE: 01-OCT-1991
 - (J) PUBLICATION DATE: 16-APR-1992
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 6: FROM 1 TO 20
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

TCTGCAGTAG CAGTCTTGC

20

(2) INFORMATION FOR SEQ ID NO: 7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Salmonella enteritidis/Salmonella typhi
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 9206197 A1
 - (I) FILING DATE: 01-OCT-1991
 - (J) PUBLICATION DATE: 16-APR-1992
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 7: FROM 1 TO 23
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 9206198 A
 - (I) FILING DATE: 01-OCT-1991
 - (J) PUBLICATION DATE: 16-APR-1992
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 7: FROM 1 TO 23
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GCTCAGAATA CAACATCAGC CAA

23

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Salmonella enteritidis*/*Salmonella typhi*

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 9206197 A1
- (I) FILING DATE: 01-OCT-1991
- (J) PUBLICATION DATE: 16-APR-1992
- (K) RELEVANT RESIDUES IN SEQ ID NO: 8: FROM 1 TO 21

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 9206198 A
- (I) FILING DATE: 01-OCT-1991
- (J) PUBLICATION DATE: 16-APR-1992
- (K) RELEVANT RESIDUES IN SEQ ID NO: 8: FROM 1 TO 21

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

AAAACAGGCT GTCCTTGTC A

21

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Salmonella enteritidis*/*Salmonella typhi*

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 9206197 A1
- (I) FILING DATE: 01-OCT-1991
- (J) PUBLICATION DATE: 16-APR-1992
- (K) RELEVANT RESIDUES IN SEQ ID NO: 9: FROM 1 TO 2387

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 9206198 A

- (I) FILING DATE: 01-OCT-1991
- (J) PUBLICATION DATE: 16-APR-1992
- (K) RELEVANT RESIDUES IN SEQ ID NO: 9: FROM 1 TO 2387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

TTAGCGTTTC TTGAGAGCTG G

21

(2) INFORMATION FOR SEQ ID NO: 10:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iii) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Salmonella enteritidis*/*Salmonella typhi*
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 9206197 A1
 - (I) FILING DATE: 01-OCT-1991
 - (J) PUBLICATION DATE: 16-APR-1992
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 10: FROM 1 TO 2387
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 9206198 A
 - (I) FILING DATE: 01-OCT-1991
 - (J) PUBLICATION DATE: 16-APR-1992
 - (K) RELEVANT RESIDUES IN SEQ ID NO: 10: FROM 1 TO 2387

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
TTTTGATACT GCTGAACGTA G

21

CLAIMS

1. A method for testing for the presence of microorganisms of salmonella serotype S. typhi comprising testing a sample of a material under investigation for the presence of a nucleic acid sequence characteristic of the genomic DNA from the region encoding for SEFA (amino acid SEQ ID No 2) or its alleles and relating the presence of any of these to the presence of that serotype.
2. A method as claimed in claim 1 wherein the sample is tested for the presence of a nucleic acid sequence characteristic of either one of SEQ ID No 1 and SEQ ID No 3.
3. A method as claimed in claim 1 wherein the sequences characteristic of the SEFA encoding region are present within the sequence that directly encodes for SEFA, as herein described in SEQ ID No 1 as bases 73 to 600 or in SEQ ID No 3 as bases 1788 to 2315.
4. A method as claimed in any one of claims 1 to 3 comprising exposing DNA or RNA derived from the material to one or more hybridization probes targeted at said sequence and relating the occurrence of specific hybridization to the presence of said serotype.
5. A method as claimed in any one of claims 1 to 3 comprising subjecting the sample to conditions under which the characteristic sequences are replicated by use of a specific sequence amplification reaction and relating the production of amplification product to the presence of S. typhi.
6. A method as claimed in claim 5 wherein the specific sequence amplification reaction is a polymerase chain reaction.
7. A method as claimed in claim 5 or claim 6 wherein the identity of any replicate sequence produced by the amplification reaction is determined by exposing it to polynucleotide hybridization probes targeted at it and relating the occurrence of specific hybridization to the presence of that sequence.

8. A method for testing for the presence of microorganisms and/or nucleic acids of salmonella serotypes S. typhi, S. enteritidis or S. dublin comprising:

(a) testing a sample of a material under investigation for the presence of a nucleic acid sequence characteristic of the genomic DNA from the region encoding for SEFA (amino acid SEQ ID No 2) or its alleles and relating the presence of any of these to the presence of one of these serotypes;

(b) testing the sample or a further sample of the material for the presence of an antigenic amino acid sequence or antibody thereto or a polynucleotide sequence encoding for such antigenic amino acid sequence, said amino acid sequence being associated with one or more of the S. enteritidis, S. dublin or S. typhi serotypes, but not found in all three, and relating the presence of this to the presence of S. typhi or S. enteritidis or S. dublin.

9. A method as claimed in claim 8 wherein step (a) is carried out by testing the sample for the presence of a nucleic acid sequence characteristic of either one of SEQ. ID No 1 or SEQ. ID No 3.

10. A method as claimed in claim 8 or 9 wherein the amino acid sequence comprises that of S. dublin p protein or S. dublin/S. enteritidis G component.

11. A method as claimed in any one of claims 8 to 10 wherein for step (b) a culture medium capable of supporting expression of SEFA by S. enteritidis or S. dublin is inoculated with a sample of the material under investigation and incubated, the resultant medium being tested for expressed SEFA.

12. A method as claimed in claim 11 wherein the culture medium consists of Peptone water and Enriched E broth, desoxycholate citrate agar, McConkey agar, Nutrient agar, Salmonella Shigella agar, Sheep blood agar, Xylose Lysine descholate, Oxoid Isosensitest agar or Oxoid Sensitest agar.

13. A method as claimed in claim 11 wherein the culture medium consists of Oxoid Isosensitest agar or Oxoid Sensitest agar.

14. A method as claimed in any one of claims 11, 12 or 13 wherein the culture is carried out at a temperature greater than 22°C.

15. A method as claimed in any one of claims 4 to 7 wherein the probes or primer sequences are selected from SEQ ID Nos 4 to 10.

16. A method as claimed in either of claims 6 and 7 wherein the primers are selected one from each of groups (A) and (B):

Group A:

SEQ ID No 4

SEQ ID No 5

SEQ ID No 6

SEQ ID No 7

Group B:

SEQ ID No 8

SEQ ID No 9

SEQ ID No 10

17. A method as claimed in any one of claims 4 or 7 wherein the probe selected from sequences of either of groups A or B described in Claim 15 provided that where the polymerase chain reaction is used the probe sequence is different to that of either of the primers used for step (b).

18. A test kit for performing a test as claimed in any one of claims 1 to 17 comprising one or both of:

(a) polymerase chain reaction probes targeted at characteristic parts of polynucleotide sequences encoding for SEFA or an epitopic part thereof and capable of initiating polymerase chain reaction production of these sequences in the presence of taq polymerase and

(b) hybridization probes targeted at characteristic parts of polynucleotide sequences encoding for SEFA or an epitopic part thereof and

18. A test kit as claimed in claim 17 further comprising one or more of:

- (i) antibodies to SEFA or an epitopic part thereof or cells capable of producing those antibodies;
 - (ii) SEFA or an epitopic part thereof in the form of cells, fimbria isolated SEFA or said part or any of these immobilised onto a surface;
 - (iii) secondary antibodies capable of specific binding to the antibodies to SEFA or to antibodies to the epitopic part thereof and
 - (vi) medium or media capable of supporting or switching off expression of SEFA by S. enteritidis and/or S. dublin or essential components for preparing such medium or media.
19. A test kit as claimed in claim 18 wherein the antibodies are immobilised on a solid carrier.
20. A test kit as claimed in claim 18 or 19 further comprising an antibody labelling agent.
21. A test kit as claimed in Claim 20 wherein the labelling agent comprises latex particles.
22. A test kit as claimed in any one of Claims 18 to 21 wherein the antibodies are in labelled form.
23. A test kit as claimed in Claim 22 wherein the components comprise the dry components for preparation of peptone water pH 7.2, peptone water pH 6.0 or a Medium B (as herein described).
24. A test kit as claimed in Claim 23 wherein the Medium B is Sensitest agar or Isosensitest agar.
25. A test kit as claimed in Claim 18 wherein the SEFA or epitopic part thereof or antibodies thereto are immobilised on a microtitre plate.

26. A test kit as claimed in claim 18 comprising hybridization probes targeted at sequences characteristic of SEQ. ID 2 or SEQ. ID No 3.

27. A test kit as claimed in claim 26 wherein the probes comprise sequences comprising sequence directly coding for SEFA.

28. A test kit as claimed in claim 18 comprising primers and probes having sequences selected from the groups (A) and (B) set out in claim 16.

INTERNATIONAL SEARCH REPORT

PCT/GB 93/00647

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 C12Q1/68; G01N33/569		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C12Q ; G01N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claims No. ¹³
A	WO,A,9 201 056 (INSTITUT PASTEUR) 23 January 1992 see page 5, line 6 - page 10, line 33; claims ---	1
A	EP,A,0 383 509 (ORTHO DIAGNOSTICS SYSTEMS) 22 August 1990 see page 2, line 45 - page 4, line 13; claims; table 2 ---	1
P,A	WO,A,9 206 198 (THE MINISTER FOR AGRICULTURE OF GREAT BRITAIN AND NORTHERN IRELAND) 16 April 1992 cited in the application see the whole document --- -/-	1
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
26 JULY 1993	06. 08. 93	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	LUZZATTO E.R.	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,A	WO,A,9 206 197 (THE MINISTER OF AGRICULTURE OF GREAT BRITAIN AND NORTHERN IRELAND) 16 April 1992 cited in the application see page 2, line 15 - page 13, line 8; claims -----	1,8

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

GB 9300647
SA 72104

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

26/07/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9201056	23-01-92	FR-A-	2664614	17-01-92
		EP-A-	0538353	28-04-93

EP-A-0383509	22-08-90	CA-A-	2009708	13-08-90
		JP-A-	2295498	06-12-90

WO-A-9206198	16-04-92	AU-A-	8656691	28-04-92
		EP-A-	0551324	21-07-93
		AU-A-	8548991	28-04-92
		EP-A-	0551325	21-07-93
		WO-A-	9206197	16-04-92

WO-A-9206197	16-04-92	AU-A-	8548991	28-04-92
		EP-A-	0551325	21-07-93
		AU-A-	8656691	28-04-92
		EP-A-	0551324	21-07-93
		WO-A-	9206198	16-04-92
